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THE FISHES OF THE MALAYSIAN FAMILY PHALLOSTETHIDAE (ATHERINIFORMES)

Tyson R. Roberts¹

ABSTRACT. The tiny fishes of the family Phallostethidae, from Malaya and Thailand, develop a large penis and differ radically in structure of the bilaterally asymmetrical priapium from the somewhat larger fishes of the more widely distributed Neostethidae, the only other family in the sub-order Phallostethoidea. A morphological characterization of the Phallostethidae is given and its features compared to those of Neostethidae. There are three species: *Phallostethus dunckeri* Regan (1913), known only from the type specimens collected in the mouth of the Muar River in Johore, Malaya; *Phenacostethus smithi* Myers (1928), known from the types and many other specimens collected in the khlongs of Bangkok and reported in this paper from Chantaburi Province in southeast Thailand; and *Phenacostethus posthon*, new species, from the Indian Ocean coast of peninsular Thailand. *Ph. posthon* and *Ph. smithi* differ considerably in morphology of the priapium and penis. Furthermore, the asymmetrical priapium in *Ph. posthon* is invariably sinistral. In all other phallostethoids, so far as known, it may be either sinistral or dextral; in *Ph. smithi* the ratio of sinistral and dextral males is near equality (Hubbs and Hubbs, 1945).

The ecology of phallostethids is described for the first time, excepting some brief remarks by H. M. Smith (1927; 1945), who seems to have confused *Phenacostethus* in the field with neostethids and perhaps with *Oryzias*. An hypothesis is offered that the selective advantage of internal fertilization in Phallostethoidea (an oviparous group) lies in permitting temporal separation of mating and spawning activities, corresponding, respectively, with periods of low water and high water in habitats subject to strong tidal fluctuations. Two trends in the reproductive biology of atheriniform fishes that might be conducive to the evolution of internal fertilization are: 1) towards eggs in which either embryonic development is slowed down or temporarily arrested, or hatching of embryos is deferred; and 2) away from expelling all ovulated eggs at once and towards expelling them in small batches or even singly.

¹ Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138.

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For translations of the papers by Aurich and Woltereck I am obliged to Miss Deborah White and Dr. Elizabeth Deichmann. Prof. George S. Myers read the paper in manuscript.

INTRODUCTION

This account of the family Phallostethidae is the first paper dealing with the fresh- and brackish-water fishes collected by me in Thailand from April 15 to July 14, 1970, and deposited in the fish collection of the Museum of Comparative Zoology. Collecting phallostethoid fishes was one of the main objectives of my fieldwork in Thailand. In addition to *Phenacostethus smithi* Myers (1928) and the new phallostethid described in this paper, large series of the neostethids *Neostethus siamensis* Myers (1937) and *Ceratostethus bicornis* (Regan, 1916) were obtained. These represent the first specimens of *Ceratostethus* recorded from Thailand, and the only specimens of *Neostethus siamensis* other than

the female holotype. (*N. siamensis* is close to, and perhaps specifically identical with, *N. lankesteri* Regan (1916), the type locality of which is the mouth of the Muar River, Johore, and Singapore.) The osteology and functional anatomy of *Cerato-stethus* will be considered in another paper.

The Phallostethoidea are small, highly specialized fresh- or brackish-water fishes in which males have a remarkable subcephalic copulatory organ, the priapium. The skeleton and musculature of this complicated bilaterally asymmetrical organ, which functions both in clasping and intromission, are derived mainly from the pelvic fins and girdle. Minor contributions come from the first pair of ribs and anteroventral part of the pectoral girdle. In females the pelvic fins are absent or vestigial. Aurich (1937) divided the Phallostethoidea into two "Familien," but gave these divisions names in subfamily form, Phallostethinae and Neostethinae. Berg (1940: 465-466) recognized them as families, Phallostethidae and Neostethidae, as did Rosen (1964: 261) and Greenwood et al. (1966: 398). Neostethidae, comprising eight genera and about 15 species, have been recorded from Thailand, Malaya, Sumatra, Borneo, and the Philippine Islands. Six of the genera — *Gulaphallus* Herre (1925), *Mirophallus* Herre (1926), *Plectrostethus* Myers (1935), *Solenophallus* Aurich (1937), *Ctenophallus* Herre (1939), and *Manacopus* Herre (1940) — are known only from the Philippines.

The Phallostethidae, even more specialized (and rarer in museum collections) than Neostethidae, comprise three species from the Malay Peninsula and adjacent parts of Thailand. *Phallostethus dunckeri* Regan (1913), the first phallostethoid to be described, is known only from the type specimens Duncker collected previous to 1904 at the mouth of the Muar River, about 20 miles south of Malacca, Johore Province, Malaya (Duncker, 1904: 171). *Phenacostethus smithi*, hitherto known only from several large series collected by H. M. Smith from khlongs in Bangkok, was obtained by me at Bangkhen (a suburb of Bangkok) and at Chantaburi, near the southeast corner of Thailand (near Cambodia). The third species, described in this paper, is from the Indian Ocean coast of Thailand. All localities where phallostethids have been collected are shown in Figure 1. The present paper presents characterizations of the family Phallostethidae and of the phallostethid species, a description of the new species just mentioned, and observations on the ecology of Phallostethidae.

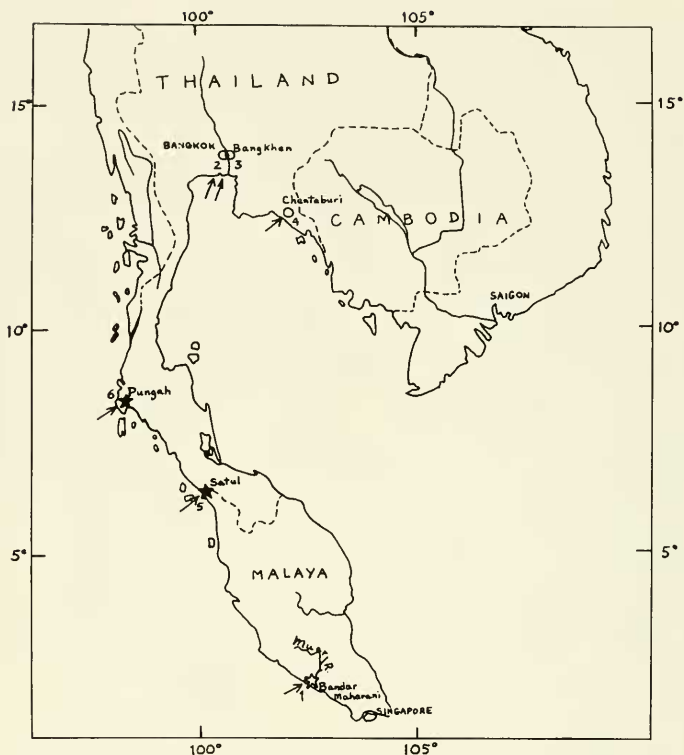


Figure 1. All localities where Phallostethidae have been collected. 1. Mouth of Muar River at Bandar Maharani (type locality of *Phallostethus dunckeri* Regan, 1913); 2. Bangkok (type locality of *Phenacostethus smithi* Myers, 1928); 3. Bangkok, a suburb of Bangkok (*Ph. smithi*); 4. Chantaburi City (*Ph. smithi*); 5. Khlong La Ngoo, 48 km NW of Satul Town, 6° 52' 30" N, 99° 48' 10" E (type locality of *Phenacostethus posthon* n. sp.); 6. Khlong Kla Sohm, 15 km S of Pungah Town, on Pakasem Road from Pungah going towards Phuket Island (type locality of *Ph. posthon* n. sp.)

Definition of the suborder Phallostethoidea and discussion of phyletic trends in the Phallostethoidea will be presented in a forthcoming paper on *Ceratostethus*.

Ph. smithi provides an Asian example of the phenomenon (of which Africa and South America provide numerous examples)

that the smallest fishes amidst the richest tropical freshwater faunas are representatives not of the dominant fish groups present, but of groups with a marginal distribution pattern. The ecology of such minute fishes, when known, usually proves to be highly specialized.

Rosen (1964) united the exocoetoids, scomberesocoids, adrianchthyoids, cyprinodontoids, atherinoids and phallostethoids in a new order, the Atheriniformes. Atherinoids and cyprinodontoids were widely separated in earlier classifications, the artificiality of which has become increasingly apparent. Such similarities as were noted between atherinoids and cyprinodontoids had usually been attributed to convergence. Having personally investigated the osteology of phallostethoids (which evidently are related to atherinoids), atherinoids, and cyprinodontoids, and reviewed much of the literature on osteology and reproductive biology of these groups, I am inclined to believe that they may be related. Some of the similarities in reproductive biology were first brought to my attention in a talk given by Neal R. Foster at the 1968 meetings of the American Society of Ichthyologists and Herpetologists.

Note on the figures. Figures 2–5, prepared with the aid of a Wild microscope and camera lucida, are based on formalin specimens. Formalin specimens of Phallostethidae are more nearly normal in appearance than alcoholic specimens, since the latter invariably undergo at least some shrinkage. In specimens that have been transferred from the original formalin fixative to 60 per cent ethyl alcohol for permanent storage, teeth protrude more from the gums, scales stand out more clearly, the membranous dome atop the head has disappeared, and the caudal peduncle is decidedly narrower.

CHARACTERIZATION OF THE FAMILY PHALLOSTETHIDAE

The following characterization of the Phallostethidae is based on my observations of *Ph. smithi* and *Ph. posthon*, including study of alizarin preparations, and on accounts of *Ph. smithi* by Myers (1928), Bailey (1936), and TeWinkel (1939), and of *Phallostethus dunckeri* by Regan (1913; 1916).

1. Slender elongate phallostethoids, very delicate, largely translucent, with deciduous scales; externally visible concentrations of melanophores restricted to the top of the braincase, middle of the

dorsum, midlateral intermuscular septum, priapium, and bases and edges of fin rays; maximum standard length about 23 or 24 mm.

2. Dorsum of head with a translucent, membranous dome.

3. Mature individuals of both sexes with a bright orange-yellow bar on caudal peduncle.

4. Branchiostegal rays 4.

5. Main "externalized" clasping bone in the priapium is the toxactinium; ctenactinium reduced or absent.

6. A greatly enlarged, oval, concave pad, or pulvinulus, slightly posterior to toxactinium.

7. Vas deferens terminating in a large penis that projects considerably from the priapium.

8. Pelvic spines or rays, if present, greatly reduced and modified beyond recognition.

9. Vas deferens highly coiled, forming a sort of epididymis.

Comments on the family characters. Neostethids, while small as fishes go, are all or almost all larger when adult than phallostethids. Most, if not all, neostethids are harder fishes than phallostethids and have relatively adherent scales. While they also are largely translucent, neostethids usually have relatively more melanophores than phallostethids. The epidermis paralleling the scale margins is often well provided with melanophores in neostethids but invariably devoid of melanophores in phallostethids. Neostethids (*Ceratostethus* and *Neostethus*), even at comparable sizes, lack a membranous dome on the dorsum of the head, or if one is present, it is not notably elevated. According to TeWinkel (1939) this region bears sensory canals in both *Phenacostethus* and *Gulaphallus*. In neostethids large sensory pores are evident in the frontal region, whereas the membranous dome in phallostethids is apparently entire.

With regard to characteristic number 3 (bright orange-yellow markings at the base of the caudal fin and sometimes at the origin of the anal fin), these are present in living specimens of *Ph. smithi* and in *Ph. posthon*. There is no way of telling whether such spots are also present in *Phallostethus*. The orangish yellow coloration, contained in chromatophores (approximately 50–75 chromatophores constituting the caudal base mark) gradually disappeared after a few weeks of preservation in formalin. Similar markings

were definitely absent in live specimens of *Neostethus* and *Ceratostethus* observed by me, nor is there any mention of such marks in the literature on Neostethidae.

Concerning character 4, Neostethidae usually have 5 branchiostegal rays. The number of branchiostegal rays in *Phallostethus* is unknown.

Characters 5–9 concern the priapium, which differs fundamentally from that of Neostethidae. In Neostethidae the main “externalized” bony clasping element, the ctenactinium, is apparently a modified pelvic fin ray or spine. The main “externalized” bony element in the priapium of phallostethids, the toxactinium, is not homologous with the ctenactinium. My observations confirm Bailey’s view (Bailey, 1936: 463, 471) that it is homologous with the pulvinular bone, one of the anteriormost internal bony elements in the priapium of neostethids. The homologies of this element are unclear, but it is almost certainly not a modified pelvic ray or spine. The ctenactinia of phallostethids, which may or may not be homologous with the elements called ctenactinia in neostethids, are greatly reduced in size. The ctenactinium of *Phallostethus dunckeri*, while relatively short, bears several “teeth” or sharp projections; these are perhaps comparable to the single curved hook present near the base of the ctenactinium in *Neostethus*. The pulvinulus of phallostethids probably functions as a pad in conjunction with the toxactinium. A homologous but much smaller pulvinulus is present in neostethids. A striking characteristic of the phallostethid priapium is the development of a large penis. In *Phenacostethus posthon* the organ is entirely smooth; in *Ph. smithi* its distal half bears a series of stiff ruffled pleats. The development of a large penis evidently occurred independently in the neostethid *Mirophallus bikolanus* (Herre, 1926, pl. 3, fig. 1). In most neostethids a complicated flap covers the opening of the vas deferens (Aurich, 1937). This flap is absent in Phallostethidae. Various bony elements in the priapium of phallostethids, including a peculiar slender element lodged in the concave side of the penis bone in *Ph. smithi*, may be homologous with pelvic rays, but the priapium of phallostethids does not bear any branched elements that obviously are relatively unmodified pelvic rays. Several branched pelvic rays of relatively normal appearance occur in the neostethids *Ceratostethus*, *Neostethus*, *Solenophallus*, and *Gulaphallus* (personal observations; Aurich [1937], TeWinkel [1939], Woltereck [1942 a, b]).

The priapium of *Phallostethus* is clearly of the same general type as that of *Ph. smithi* and *Ph. posthon*. In *Phallostethus* the toxactinium and pulvinulus are very similar to these structures in *Phenacostethus*. It is likely that *Phallostethus* develops a large penis. The penis in phallostethids, like the ctenactinium of neostethids, only reaches its full development in the largest males.

Regan (1916: 22) hypothesized that in *Phallostethus* the toxactinium grips the female under the chin or is held in her mouth, while the serrated edge of the ctenactinium gives a firm hold on the pectoral region in front of and on the far side of the genital orifice, in order that the seminal papilla could be placed against it or introduced into it. In *Neostethus* he hypothesized that the female is held across the back of the head by the ctenactinium, the anterior descending part of which lies on the side of the female away from the male. Copulation has yet to be observed in *Neostethus* or in any phallostethids. It has been observed only in the neostethid *Gulaphallus mirabilis*. In this species the female is held across the back of the head by the ctenactinium; the "second ctenactinium" of *Gulaphallus mirabilis*, which actually is an externalized pelvic bone, apparently rests or presses against the female's opercular region on the side next to the male (Villadolid and Manacop, 1934: pl. 5, fig. 2). The reduced ctenactinium of Phallostethidae could hardly function in the same manner as the elongate ctenactinium characteristic of all neostethids. It may be that the toxactinium is held, not under the female's chin or in her mouth, but atop the front of her head.

In *Phallostethus* (Regan, 1916: 19, fig. 14) the vas deferens is highly coiled within the abdominal cavity to form a sort of epididymis. TeWinkel (1939) reports a similar coiling of the vas deferens in *Ph. smithi*. In *Neostethus lankesteri* (Regan, 1916: 10, fig. 6) and in *Gulaphallus mirabilis* (Villadolid and Manacop, 1934: pl. 3, fig. 4) the vas deferens is unconvoluted from its origin on the testis to where it enters the priapium, then forms an expanded loop inside the priapium. The sperm of *Neostethus lankesteri* (Regan, 1916: 13, fig. 9) are concentrated into "spermatophores," more properly called spermozeugmata (Nielsen et al., 1968: 248). Regan (1916: 19) stated that *Phallostethus* evidently did not produce "spermatophores" like those of *Neostethus*. In mature *Neostethus* and *Ceratostethus* (personal observation) the posteriormost portion of the priapium is

sometimes greatly swollen with closely packed, adherent small vesicles that presumably are spermozeugmata. The priapium apparently does not become similarly swollen in phallostethids.

Insofar as can be determined from examination of the bones only, the contributions of the first pair of ribs and of the shoulder girdle to the priapium is the same in Phallostethidae and Neostethidae.

THE SPECIES OF PHALLOSTETHIDAE

Phallostethus dunckeri Regan 1913

Phallostethus dunckeri Regan, 1913: 550, figs. 1-4 (original description; types from mouth of Muar River at Bandar Maharani, Johore, Malaya; soft anatomy). — Regan, 1916 (soft anatomy, histology, osteology, comparison with *Neostethus*).

This species is known only from the specimens collected by G. Duncker at the mouth of the Muar River at Bandar Maharani, Johore, Malaya. The following statements are based on the two accounts of this species by Regan (1913, 1916). It is characterized by an exceptionally long anal fin, with 26-28 elements (eight to ten rays more than are found in phallostethoids with the next highest number of anal fin elements), an anal base about 30 per cent of the standard length (compared to anal base 20-25 per cent of standard length in all other phallostethoids) and origin of anal considerably nearer to snout tip than to end of hypural fan (anal origin slightly to considerably nearer to end of hypural fan than to snout tip in all other phallostethoids), and by a serrated ctenactinium (Regan, 1916: fig. 13). Regan did not have very many specimens (some were used for histological preparations or cleared in oil of cloves for bone study). Dr. P. H. Greenwood informs me that the British Museum (Natural History) has four specimens (types?) of *Phallostethus dunckeri* in rather poor condition. Although Regan's specimens of *Phallostethus dunckeri* (at 23-29 mm in total length) are larger than *Phenacostethus*, and the males figured by him have well-developed priapia, perhaps the penis is not fully developed in them. This seems likely, because in both species of *Phenacostethus* the largest females are only about one-half to one millimeter longer than the largest males, whereas Regan's largest female of *Phallostethus* is six millimeters longer than his largest male. In *Phenacostethus* the penis is fully formed only in the largest males.

The second dorsal fin of *P. dunckeri* has eight to ten elements (a number found in some Neostethidae); the highest number in *Phenacostethus* is seven. It is unknown whether *Phallostethus* has a first dorsal fin. Regan did not mention its presence, but he overlooked the first dorsal in his material of *Neostethus lankesteri* and *Ceratostethus bicornis*. *Phallostethus* probably has a small spinous first dorsal fin, but the absence of one would not be unique among Phallostethoidea: the first dorsal is lacking in the neostethids *Mirophallus bikolanus* Herre (1926), (Herre, 1942: 141; Myers, 1937: 142) and *Solenophallus thessa* Aurich (1937: 265).

Phenacostethus smithi Myers 1928

(Figures 2, 4, 6)

Neostethus lankesteri (not of Regan) Smith, 1927: 353-355 (misidentification).

Phenacostethus smithi Myers, 1928: 6, figs. 1 and 2 (original description; types from Bangkok; holotype in American Museum of Natural History). — Bailey, 1936 (osteology). — TeWinkel, 1939 (soft anatomy). — Smith, 1945: 475 (Bangkok; synonymizes *Ph. thai* Fowler; ecology).

Phenacostethus thai Fowler, 1937: 219; figs. 189, 190 (original description; Bangkok; holotype in Philadelphia Academy).

Material studied. MCZ 47055, 13 specimens, five females 13.7-14.8 mm and eight males 13.6-14.9 mm (three with toxactinium arising on left side, five on right side), from Khlong Bangkhen at bridge on Nzarm Wong Wan Road, a few km west of Kasetsart University, Bangkhen, Bangkok, Thailand, 30 April 1970; MCZ 47299, 20 specimens, three females 13.4-15.6 mm and 17 males 12.7-14.9 mm (12 with toxactinium arising on right side, five on left side), from Khlong Kee Nawn, behind Catholic church in Chantaburi City, Chantaburi Province, Thailand, 5 May 1970.

Myers' figure 1 of *Ph. smithi* does not show the membranous dome of the dorsum of the head (Fig. 2); it is likely to be shrunken in alcoholic specimens. The adult male in Myers' figures 1 and 2, at 13.5 mm in standard length, does not have the penis as fully developed as in my specimens 14.3 (Fig. 2), 14.1 (Fig. 4), and 14.5 (Fig. 6) mm in standard length. In these specimens the penis is much larger, and its distal end bears a series of a half-dozen or more crenulated radial folds or extensions resembling a

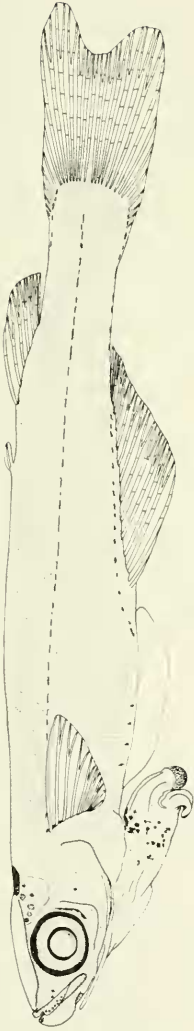


Figure 2. *Phenacostethus snithi* Myers (1928), 14.3-mm sinistral male, MCZ 47299 (formalin).

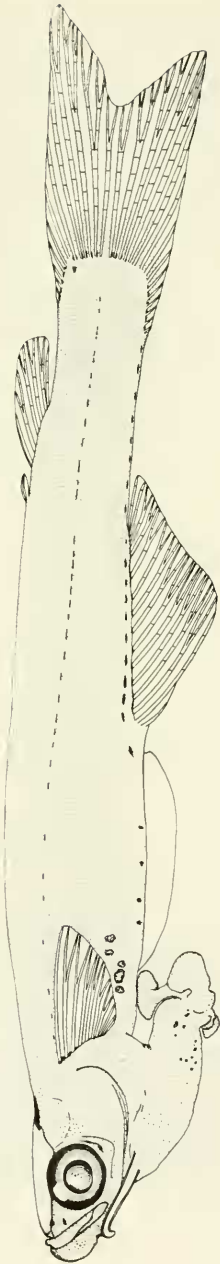


Figure 3. *Phenacostethus poshoni* n. sp., 17.3-mm male paratype, MCZ 47301 (formalin).

set of ruffled lace cuffs (best shown in Fig. 4). These folds can be seen in one of Bailey's anatomical figures based on material of *Ph. smithi* collected by Hugh M. Smith. Direct comparison of *Ph. smithi* collected by Smith in Bangkok (deposited in the fish collection of the College of Fisheries, Kasetsart University, and in the MCZ fish collections) with my material from Bangkhen and Chantaburi indicates that only one species is involved. Live specimens of *Ph. smithi* of both sexes from Bangkhen and from Chantaburi had a bright orange-yellow, anteroventrally-posterodorsally oriented bar on the lower half of the caudal peduncle. This bar disappeared from specimens kept a few weeks in formalin. The color was due to about 50–75 chromatophores. There is a small, shiny blue spot over the brain (well behind the eyes), and a very small silvery spot in the middle of the eyes' dorsal surface.

Alizarin preparations reveal the first dorsal fin consists of a single tiny spine, as indicated by Myers. Most specimens are missing many scales. Head scaleless. Abdominal keel scaleless in both sexes (abdominal keel scaled in females of *Ceratostethus bicornis*). Scales on body cycloid, number of scale rows corresponding closely with the number of myotomes. Teeth in both jaws in a single series; medial portion of premaxillary with about eight sharp conical teeth, lateral (expanded) margin of premaxillary with about seven to nine slightly larger conical teeth; medial portion of dentary with about a dozen conical teeth opposing but much smaller than those on medial portion of premaxillary.

For counts of fin elements and vertebrae based on alizarin preparation see Table 1.

Phenacostethus posthon, new species
(Figures 3, 5, 7)

Holotype. MCZ 47300, a 16.7-mm male from Khlong Kla Sohm about 15 km southwest of Pungah Town, where it is crossed by a bridge on the Pakasem Road (between Pungah Town and Phuket), Pungah, Thailand. 29 June 1970.

Paratypes. MCZ 47301, 58 specimens, comprising two immatures, 11.9 and 12.0 mm, 22 females, 12.3–17.7 mm, and 34 males 12.7–17.0 mm (of which two males, 15.5 and 16.5 mm, are cleared and stained), same data as holotype; MCZ 47302, 20 specimens, comprising three immatures 9.6–12.0 mm, 10 females 12.7–18.0 mm, and 7 males 12.9–16.7 mm, from Khlong Langu

at Langu Town, 48 km northwest of Satul Town ($6^{\circ} 52' 30''$ N, $99^{\circ} 48' 10''$ E). 23 June 1970.

The most obvious differences between this species, from the Indian Ocean coast of Thailand (Fig. 1), and *Ph. smithi* involve the priapium and the position of the dorsal fin. Granted that both species have the characteristic priapial elements of the family Phallostethidae, the priapium is, in fact, so different in the two species that the question may even be raised as to whether separate generic status is indicated.

Differences in the priapium of the two species involve external morphology of the penis, skeleton of the penis, ctenactinium, toxactinium, and the nature of the laterality of the priapium itself. In *Ph. smithi* 1) the distal portion of the penis is ruffled (see description above); 2) the penial skeleton includes a large papillary bone with a slender penial bone lodged in its concave surface (Fig. 6; see also Bailey, 1936: 3 and 4); 3) the ctenactinium is relatively large and externally evident; 4) the toxactinium is relatively slender and gently curved; and 5) the priapium itself may be either sinistral or dextral. In *Ph. posthon*, on the other hand, 1) the penis is smooth; 2) the penial skeleton has a large papillary bone but the penial bone is absent; 3) the ctenactinium, if it is present at all, is reduced and hardly detectable externally; 4) the toxactinium is stouter and distinctly more sharply curved; and 5) the priapium is invariably sinistral (toxactinium arising on left side) in the material examined.

Ph. posthon and *Ph. smithi* of both sexes can be distinguished at a glance by the position of the first dorsal fin relative to the anal fin base. In *Ph. posthon* the first dorsal (which has a single spine as in *Ph. smithi*) originates slightly posterior to the base of the last ray in the anal fin; in *Ph. smithi* it originates over the middle of the anal fin base. The first dorsal fin is somewhat closer to the second dorsal origin in *Ph. posthon* than in *Ph. smithi*. *Ph. posthon* is more elongate (depth of body at anal fin origin about 7, vs. 6 in *Ph. smithi*) and evidently a larger species. The average and maximum sizes of specimens in my two samples of *Ph. posthon* are definitely larger than in my two samples of *Ph. smithi* (which include specimens larger than those previously recorded). The largest male and female specimens of *Ph. smithi* are, respectively, 14.9 and 15.6 mm; of *Ph. posthon*, 17.0 and 18.0 mm (standard lengths). The largest specimens (both sexes) had a bright orange-yellow bar on the caudal peduncle, as in *Ph. smithi*,

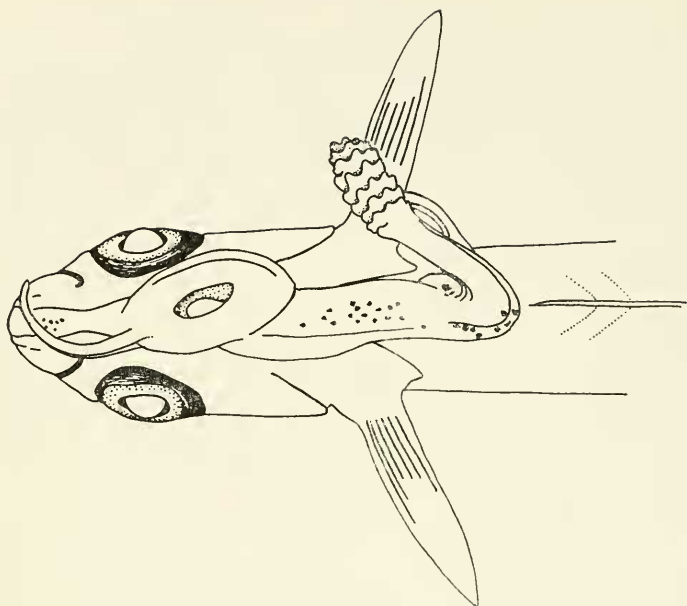


Figure 4. *Phenacostethus smithi* Myers (1928), ventral view of priapium, 14.1-mm specimen, MCZ 47055 (formalin).

and also a smaller orange-yellow bar on the body next to the anal fin origin.

As in *Ph. smithi*, there appears to be a one-to-one correspondence between the scale rows and the myotomes. The thin scales are extremely difficult to see in specimens under alcohol or water; and most specimens are missing many scales. I find in several specimens eight oblique scale rows between dorsal midline and anal fin origin, and six oblique scale rows between dorsal fin origin and anal base. Shape of jaw bones and disposition of teeth as in *Ph. smithi*.

For fin and vertebral counts based on alizarin preparations of *Ph. posthon* see Table 1. *Ph. posthon* seems to have, on the average, one less ray in the dorsal fin (verified by counts of unstained specimens) and one more vertebra than *Ph. smithi*.

Right- and left-handedness in the priapium of Phallostethidae.

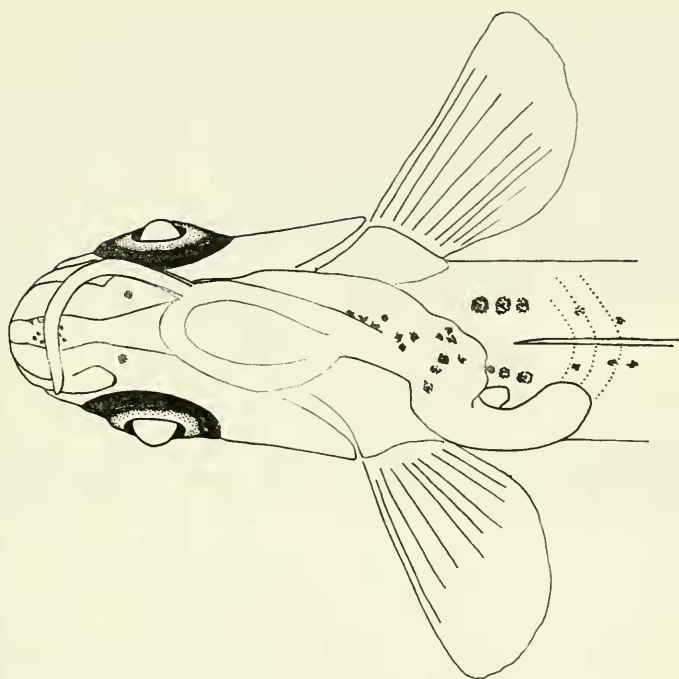


Figure 5. *Ph. posthon* n. sp., ventral view of priapium in 16.9-mm paratype, MCZ 47301 (formalin).

That the priapium of *Neostethus lankesteri* may be either right- or left-handed is stated by Regan (1916: 5). Regan did not specifically state that the priapium of *Phallostethus* may be either way, but in a general discussion of the priapium as compared to copulatory organs of other fishes, he refers to its being either dextral or sinistral (Regan, 1916: 23). Regan's figures of *Phallostethus* depict both right-handed and left-handed males. It is conceivable that one or more of them might be reversed images, the images being reversed either by the illustrator or by the printer, so the figures cannot be taken as definite proof that *P. dunckeri* may be either left-handed or right-handed. Concerning *Phenacostethus smithi*, Hubbs and Hubbs (1945: 294) found that in 334 males (from material collected by H. M. Smith at Bangkok)

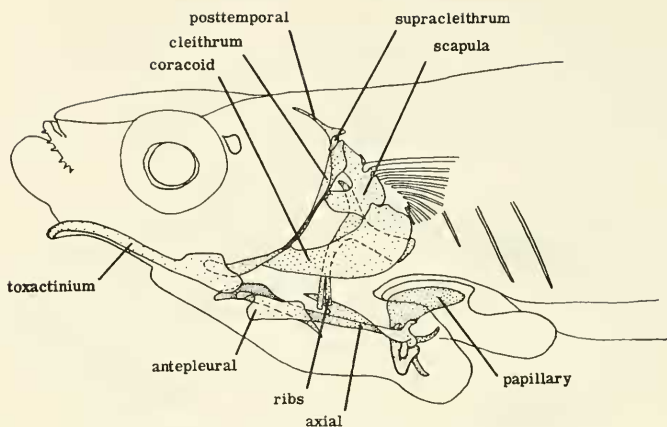


Figure 6. *Ph. smithi* Myers (1928), skeleton of priapium and pectoral fin in 14.5-mm specimen, MCZ 47301. Terminology of priapial elements according to Bailey (1936).

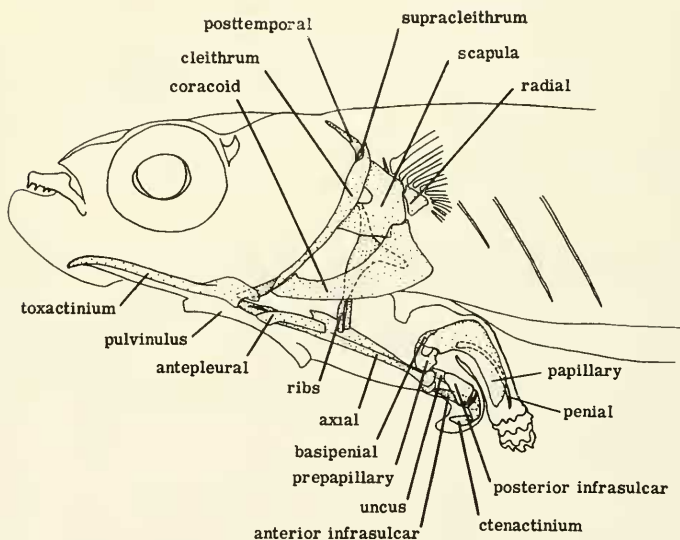


Figure 7. *Ph. posthon* n. sp., skeleton of priapium and pectoral fin in 16.5-mm paratype, MCZ 14301. Priapial elements of uncertain homology with those in *Ph. smithi* are unlabelled.

the aoproctal side was the left side in 155 and the right side in 179. The deviation from equality is statistically insignificant. In my samples from Bangkhen and from Chantaburi (too small for statistical analysis) I find both left- and right-handed males. In *Phenacostethus posthon* the toxactinium arises on the left side in every male in which the priapium is sufficiently developed to have a toxactinium (27 specimens from Pungah and five from Satul). To my knowledge this is the only phallostethoid in which the sidedness of the priapium appears to be fixed. Females of Phallostethidae are bilaterally symmetrical, the genital openings lying in the middle of the throat.

ECOLOGICAL OBSERVATIONS

The only ecological information Regan had about *Phallostethus dunckeri* was that it came from brackish water from the Muar River at Bandar Maharani. Duncker also collected *Neostethus lankesteri* in the same general locality.

Smith (1927; 1945: 476) made brief ecological observations on *Phenacostethus smithi* but in retrospect it seems that he sometimes confused this little fish in the field with *Neostethus* or *Ceratostethus* and perhaps even with *Oryzias*. I did not observe either phallostethids or neostethids at the surface, and feel that they generally keep well below the surface. Smith's remarks (that *Phenacostethus* "normally remain at or close to the surface, where they would be difficult to see were it not for a glistening yellow area on the top of the head") sound more like *Oryzias*. The glistening area on the top of the head in phallostethoids is relatively small and bluish, compared to the large yellowish glistening area in *Oryzias*, which is comparable to the striking head spots in rivuline cyprinodontids of the genera *Epiplatys* and *Aplocheilus*. In any event, Smith records that *Ph. smithi* abounds in freshwater pools, ditches and smaller canals in the Bangkok region, living in water that is nearly always muddy or turbid, and that the species is oviparous (although spawning was not observed); the egg-bearing and spawning periods are protracted, corresponding with the rainy season, and may extend from May to December. Small numbers were maintained in aquaria for a month by the daily introduction of fresh ditch or canal water to provide food in the form of minute crustaceans, protozoans, worms, etc. Smith found the larvae of anopheline mosquitoes much too large for *Ph. smithi* to ingest.

Undoubtedly *Ph. smithi* once lived in innumerable khlongs and ditches in Bangkok which are today so polluted that they are inhabited only by the hardiest air-breathing fishes such as *Anabas testudineus*. In Khlong Bangkhen, a few kilometers from Kasetsart University, I found *Ph. smithi* in association with a variety of primary and secondary freshwater fishes, principal among which were cyprinids mainly of the genus *Rasbora*, *Dermogenys*, *Xenentodon*, *Oryzias*, *Gobiopterus chuno* (a small translucent goby), young *Fluta*, and *Chaudhuria*. The *Phenacostethus* were mostly in a little backwater, close to the main current in the khlong, in very turbid water. I was unable to taste any salt in the water. At Chantaburi City, *Phenacostethus* was collected in a turbid ditch or small khlong, Khlong Kee Nawn, behind the Catholic church. There were a number of houses along the khlong at this point, and considerable rubbish had been thrown into it, so collecting efforts were confined to dip-netting for *Phenacostethus*. *Dermogenys*, *Brachygobius* and *Gobiopterus* were collected incidentally. About two kilometers downstream, where the khlong traversed a large open field, we found minnows such as *Oxygaster*, *Esomus* and *Rasbora* (but no *Phenacostethus*) which indicates the water in the khlong was not brackish.

At Satul and at Pungah *Ph. posthon* was obtained along the margins of khlongs or tiny branches of khlongs in which the water was swiftly flowing, highly turbid, and probably completely fresh. A few of the largest females (collected June 1970) were replete with ripe ova. Both localities were at places fully subject to tidal fluctuations, but far upstream from branches having water the least bit salty to the taste. At Khlong Langu in Satul Province the water level was undoubtedly high because of heavy rains for the preceding 12 hours or more; *Phenacostethus* were collected in the khlong both on the falling tide and rising tide. No primary freshwater fishes were obtained. Species in the khlong where *Ph. posthon* was collected included *Oryzias*, *Dermogenys*, *Chanda*, *Gobiopterus*, *Butis*, and *Tetraodon*. In Khlong Kla Sohm, near Pungah, *Ph. posthon* was collected in a narrow side channel more or less uniformly 3–4 feet deep, swiftly flowing, with hard-packed mud bottom, well shaded by Nipa palm and mangrove. *Oryzias*, *Dermogenys*, *Gobiopterus*, *Periophthalmus*, *Tetraodon*, and young *Scatophagus* were the only other fishes collected or observed in this channel.

In contrast, on the more numerous occasions when I obtained either *Neostethus* or *Ceratostethus* in Thailand, the water was usually brackish or even very salty to the taste. At one locality *Neostethus* and *Ceratostethus* were collected together in large numbers but neither were collected in association with *Phenacostethus*. I did not find *Neostethus* or *Ceratostethus* in association with primary freshwater fishes except on one occasion (afternoon, 11 July 1970) when *Ceratostethus* was found in a khlong (strongly influenced by tides) about midway between Bangkok and Samutsakorn, in association with *Toxotes*, *Dermogenys*, *Rasbora* and *Eso-mus*. The water was more or less fresh to the taste. A number of dead *Eso-mus* were carried by the current of the khlong, and a *Rasbora* or two may have been amongst them. I thought at the time that mortality of these minnows was perhaps due to incursion of salt water. The dead fish were carried by the outgoing tide.

My impression is that, in Thailand, phallostethids occur in water that is turbid and fresh; and neostethids in water that is turbid and brackish or even quite salty. I would guess that at some of the seven localities where I collected neostethids the water was at least a third and perhaps one-half or more as salty as sea water.

The only phallostethoid in which mating and egg-laying have been observed (but not in sequence) is the neostethid *Gulaphallus mirabilis* Herre (Villadolid and Manacop, 1934). It seems probable that all phallostethoids are oviparous. No females carrying embryos have been observed. It is unclear how much time elapses between copulation and egg-laying in *G. mirabilis*, or whether several egg-layings follow a single copulation. Judging from the range in size of young individuals found with adults in my collections of phallostethoids, I suspect that in Thailand species some reproduction goes on throughout the year. It seems likely that reproductive peaks occur towards the latter part of the rainy season (November and December).

The distribution of phallostethoids is marginal to the rich East Indian marine and freshwater fish faunas. They do not occur in the sea, nor do they penetrate very far into fresh water where there is a continental fauna of primary freshwater fishes. The Phallostethidae penetrate further into waters inhabited by primary freshwater fishes than any other phallostethoids, but they do not

get very far inland. Usually the canals or creeks they inhabit are strongly influenced by tides, and thus the faunal composition (with regard to fishes at any rate) is subject to considerable temporal variation, involving retreat of primary freshwater fishes and invasion of brackish-water forms, and vice versa. In places where the tidal changes are greatest, these invasions and counterinvasions would be a daily event, varying of course with the extent in the tides. In other places they might occur only during the strongest tides. *Gulaphallus* on the island of Luzon probably pass their entire lives in fresh water—where no primary freshwater fishes occur.

DISCUSSION

Selective advantage of internal fertilization in phallostethoids. One usually associates copulatory organs in teleostean fishes with viviparity, as in Poeciliidae, Embiotocidae, and Brotulidae. It is a striking fact, however, that several groups of oviparous teleosts normally have internal fertilization. Among these are some Cottidae, some Scorpaenidae, probably some Clinidae, and probably some glandulocaudine characids. According to Nelson (1964) the evolution of internal fertilization in the oviparous glandulocaudine characids of tropical South America appears to be a response to well-marked wet and dry seasons. The presumed advantage of a temporal separation in mating and spawning is as follows: mating occurs during the dry season, when populations are crowded together in small pools, food is scarce and conditions for survival are generally unfavorable; spawning, on the other hand, occurs during the height of the rainy season, when the population is maximally dispersed (so much so that males and females may no longer be together) and conditions for survival of the young are optimum (abundance of food, well-oxygenated waters, access to areas where most predators are too large to enter). A very similar set of conditions may apply to the evolution of internal fertilization combined with oviparity in phallostethoids, with temporal separation of mating and spawning corresponding, respectively, with periods of low water and high water. This hypothesis should be extended to the oviparous atheriniform fishes *Horaichthys setnai* Kulkarni (1940) and *Tomeurus gracilis*, males of which have independently evolved excessively complicated copulatory organs through modification of the anal fin. *Tomeurus* occurs in brackish water along the northeastern coast of South

America, including the mouth of the Amazon River, *Horaichthys* in brackish water along the Bombay and Kerala coast of India. The sperm in *Tomeurus* are transmitted in spermozeugmata (Nielsen, et al., 1968: 253), as in the viviparous poecilioids (the nature of the phyletic relationship between *Tomeurus* and poecilioids is unclear; they probably are closely related). *Horaichthys*, on the other hand, is among the very few teleosts known to have a true spermatophore (Kulkarni, 1940; Nielsen, et al., 1968). (In true spermatophores the sperm are enclosed in a capsule. A spermozeugma is an unencapsulated group of sperm held together by a mucoid substance.)

Trends in the reproductive biology of atheriniform fishes that might be conducive to the evolution of internal fertilization. When oviparous fishes with internal fertilization exhibit a marked delay between mating and spawning, it may be presumed that fertilization actually occurs just before spawning, the sperm having been stored. Storage of sperm and delayed fertilization evidently characterize at least some viviparous fishes, such as those poecilioids (including forms with superfoetation and forms without it) in which females are capable of producing up to four or five successive broods after a single mating session. It should be noted, however, that the eggs of a number of atheriniform fishes with external fertilization normally exhibit arrested embryonic development or delayed hatching. A lengthening of the period between fertilization and hatching, particularly if it could be subjected to hormonal control, might be highly preadaptive to the evolution of internal fertilization with delayed spawning. While there is no evidence of arrested embryonic development or delayed hatching in atheriniforms with internal fertilization, it seems worthwhile to review briefly what is known about these phenomena in forms with external fertilization.

Wourms (1967) found that the eggs of annual cyprinodont fishes of the subfamily Rivulinae (*Cynolebias*, *Pterolebias*, *Rachovia*, *Nothobranchius*, and *Austrofundulus*) are subject to developmental arrest at one or more stages. In *Austrofundulus myersi* dispersion of amoeboid blastomeres occurs early in development (stages 19–22) so that there is no embryo or aggregation of cells. This is followed by a slow reaggregation of cells and resumption of development. This arrested phase (Diapause I) is facultative, and also occurs in other annuals. Obligate developmental arrests

in annuals were found in presomite embryos (stage 33, Diapause II) and in prehatching embryos (stage 44, Diapause III). The duration of these phases is rather variable. Wourms suggested that "the net effect of a developmental system which can undergo diapause of variable duration at several stages is to generate a wide distribution of eggs in any single developmental stage, and to make the transition from stage to stage a variable phenomenon. The continued survival of the population is ensured in spite of climatic cycles which are variable in periodicity and intensity" (Wourms, 1967: 3411). The eggs of annual cyprinodonts have exceptionally hard chorionic membranes. Harrington (1959) reported delayed hatching in stranded eggs of marsh killifish, *Fundulus confluentus*, a member of the cyprinodontid subfamily Fundulinae, and Jones (1944) reported delayed hatching in *Oryzias melastigma*, a member of the cyprinodontoid family Oryziatidae (see Rosen, 1964, for a characterization of this family). In *O. melastigma* hatching normally occurs in 8–14 days, yet can be delayed up to six weeks; hatching can be induced by adding water of lower salinity. The only noncyprinodontoid fishes in which delayed hatching has been reported as a normal phenomenon are one or two members of the atherinid subfamily Atherinopsinae, including the grunion, *Leuresthes tenuis*. In this species, "spawning begins just after turning of tide during the lower (bright moon) series of high tides, but somewhat later than turn of tide during the higher (dark moon) series of high tides. Thus the eggs are always deposited near the same point on the beach profile so that after 2 weeks the lower series of high tides washes out the eggs deposited during the higher series, and *vice versa*. During the interim of low tides, the eggs are above tide level in moist sand 4 inches below the surface" (Harrington, 1959: 434–435, after Thompson and Thompson, 1919; Clark, 1925). Grunion eggs presumably have a highly protective covering. It seems likely that more atheriniform fishes will be found with either delayed embryonic development or else deferred hatching (with viability of embryos sustained far beyond the usual incubation periods). The outer covering of the egg in many atheriniform fishes is highly protective. These reproductive features obviously could be advantageous to fishes such as cyprinodontoids, which generally exploit niches in ephemeral waters (Foster, 1967).

Finally, it should be noted that Laale and McCallion (1968) experimentally induced developmental arrest in *Brachydanio rerio*,

a cyprinid. Exposure to extracts from *Brachydanio*, frog, or chick embryos caused *Brachydanio* embryos at stages 17–18 to stop developing. Returned to buffered water, the embryos resumed normal development and hatched. Laale and McCallion suggested that the inhibitory factor might be a nuclease.

Foster (1967) suggested that in cyprinodontoids the shift from expelling and fertilizing all of the ovulated eggs in one continuous spawning act to expelling and fertilizing them singly or in small batches greatly increased the amount of courtship behavior per fertilized egg and thereby the impact of sexual selection. The habit of expelling a few eggs at a time is also found among atherinoids (see Breder and Rosen, 1966) and is apparently characteristic of phallostethoids (Villadolid and Manacop, 1934, for *Gulaphallus mirabilis*; Myers, 1935, for *Plectrosetheus palawanensis*), *Horaichthys* (Kulkarni, 1940), and *Tomeurus* (Myers, 1947: 8–11; Breder and Rosen, 1966: 341–343). Whereas in many atheriniforms the tendency to deposit a few eggs at a time seems to have led to the evolution of prolonged courtship, in other lines it may have led to internal fertilization. Supposing females carry a fair number of eggs but lay only a few at a time, males that can impregnate the female and fertilize all of the eggs at once have an obvious selective advantage over males that can fertilize only a few eggs at a time. Even more to the point, there may be little to prevent the sperm from getting into the oviduct and fertilizing eggs retained inside the female. Thus, while fertilization normally is external in *Oryzias*, instances of *O. latipes* females with internally fertilized eggs (developing embryos) do occur (Amemiya and Murayama, 1931). *Oryzias* evidently is ancestral to *Horaichthys*.

Table I.

	Second Dorsal	Anal	Caudal	Pectoral	Vertebrae
<u>Ph. smithi</u> (MCZ 47299)	$4\frac{1}{2}$ ($5\frac{1}{2}$?)	$12\frac{1}{2}$	7 6 - 5 10	1 9	14 + $20\frac{1}{2}$
" "	$4\frac{1}{2}$ ($5\frac{1}{2}$?)	$13\frac{1}{2}$	8 4 - 6 9	1 9	14 + $19\frac{1}{2}$
<u>Ph. posthon</u> (MCZ 47301)	$3\frac{1}{2}$ ($4\frac{1}{2}$?)	$12\frac{1}{2}$	8 4 - 4 (5 ?) 10	1 8	15 + $20\frac{1}{2}$
" "	$3\frac{1}{2}$	$12\frac{1}{2}$	8 4 - 4 (5 ?) 11	1 9	16 + $19\frac{1}{2}$

Fin counts and vertebral numbers from alizarin stained and cleared male specimens of the two species of *Phenacostethus*. The distal end of the second element in the dorsal fin is not well developed (perhaps decalcified by maceration) so it is difficult to determine whether it is branched or unbranched; similarly, in some specimens it is difficult to determine the condition of the principal ray next to the outermost principal ray in the lower lobe of the caudal fin.

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